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Quessada-Vial, Audrey; van Oijen, Antoine M.

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# How DNA-repair proteins find their targets

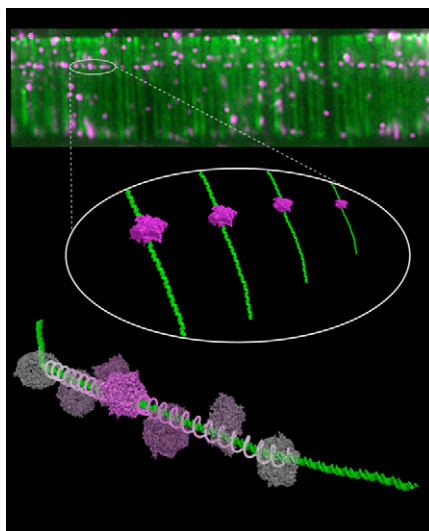
Audrey Quessada-Vial and Antoine M. van Oijen<sup>1</sup>

Zernike Institute for Advanced Materials, Groningen University, 9747 AG Groningen, The Netherlands

Genomic DNA is subject to damage at such high frequencies that only with efficient DNA-repair pathways genomic stability is maintained. Research efforts of many groups over several decades have revealed the salient properties of the various DNA-repair mechanisms, but it has largely remained unclear how damage sites are localized in genomic DNA. In PNAS, Gorman et al. (1) unveil insights into the DNA-repair process called mismatch repair (MMR), which is responsible for the repair of incorrectly paired DNA bases. They use a combination of single-molecule fluorescence imaging and nanofabrication to study how different search mechanisms are used in eukaryotic MMR to find mismatches.

The very first step of any attempt to repair damage that is highly localized to one specific position on the DNA, most often to just one base, consists of the formidable task of finding this site amid billions of bases of undamaged DNA. Often time constraints also play an important role, for example when damage needs to be corrected before replication takes place to prevent stalling of the replication machinery, or the errors irreversibly being propagated to progeny cells. Solely relying on 3D diffusion has long been thought to be insufficient for the DNA-repair process to win this race against the clock. It was proposed a number of decades ago that the search of a protein for a specific site on DNA (be it a repair protein finding lesions, transcription factor binding to promoters, or any other DNA-binding protein that needs to associate with a unique and specific position) could be drastically sped up by combining 3D diffusion through solution with short stretches of one-dimensional (1D) search along DNA (2). In such a picture, the protein would randomly bind to a position on DNA from solution, scan around the DNA in a 1D, random walk-like fashion, dissociate to rebound at another position, and repeat this cycle until the target site is found.

Several molecular scenarios have been proposed, and experimentally confirmed, that describe the molecular details of how a protein can move along DNA in a 1D fashion. The first, sliding, can be best described as the protein scanning the DNA while maintaining continuous electrostatic contact with the backbone charges of the duplex. In such a scenario, the protein is rapidly moving back and



**Fig. 1.** MutS $\alpha$  searching for the mismatch site. The DNA curtain is stretched by a laminar flow. MutS $\alpha$  is tagged with a quantum dot and visualized by total internal reflection fluorescence microscopy. Before binding to the mismatch, the protein can diffuse three-dimensionally or scan the DNA backbone one-dimensionally while rotating around the duplex.

forth while tracking the helical pitch of the DNA and thus rotating around the DNA. The second, hopping, involves the protein undergoing many rapid cycles of microscopic association and dissociation events onto and from the DNA. Although the protein does not move along the DNA during the fleeting moments it is bound to the DNA, it will be subject to 3D diffusion while transiently unbound and thus will likely rebound at a slightly different position. Many of such cycles in rapid succession will also cause the DNA to search one-dimensionally along the DNA in a random walk-like fashion. Finally, it has been proposed that many such DNA-searching proteins may switch DNA strands during their search. By binding to another, nearby DNA molecule using a mechanism similar to a monkey switching tree branches, such a so-called intersegmental transfer process may allow a protein to maximize its time spent performing a 1D search while allowing it to rapidly sample many different stretches of DNA that are close in real space but far apart in sequence space. Although these mechanisms were proposed several decades ago and their existence demonstrated by many elegant biochemical experiments, it has not been until recently that the direct observation of individual proteins moving along DNA

using single-molecule fluorescence microscopy has allowed the study of the full details of these processes and their roles in various physiologically relevant search challenges.

In MMR, the first step of the process involves a protein called MutS finding and binding to the mismatch, followed by the recruitment of MutL. MutL $\alpha$ , the eukaryotic homolog of MutL, possesses an endonucleolytic activity that allows it to cleave the DNA strand containing the error (3). Subsequently, the portion of the DNA strand between the mismatch and the cleavage is degraded (including the mismatch) and resynthesized. Importantly, the repair system needs to be able to degrade the correct portion of the DNA strand containing the mismatch, thus requiring the system to relate the position of the mismatch to that of the cleavage site. It has been long debated how the post-replicative DNA MMR process retains and transfers this information, and three main models have been proposed: (i) a translocation model that describes MutS as a motor protein that can only translocate in one direction along DNA (4); (ii) the molecular switch model, in which the ATP binding by MutS converts it into a sliding clamp that engages the cleavage site in random walk-like fashion, even though this model does not readily explain how the directionality is maintained (5); and (iii) the static transactivation model, which predicts that MutS on the mismatch forms a DNA loop connecting the mismatch with the cleavage site (6). Using single-molecules fluorescence methods, Cho et al. (7) visualized the different diffusion processes that MutS undergoes before and after the mismatch binding and demonstrated that the molecular switch model is the most likely model. In PNAS, Gorman et al. (1) not only confirm these results but also take a significant step further by studying the behavior of the MutS $\alpha$ /MutL $\alpha$  complex and thereby obtain significant insight into the different methods of search used by these proteins during the various steps of the repair process.

Gorman et al. (1) report the visualization of individual, quantum-dot tagged MutS $\alpha$

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<sup>1</sup>To whom correspondence should be addressed. E-mail: a.m.van.oijen@rug.nl.

and MutL $\alpha$  proteins on stretched DNA molecules. The authors use a method they previously developed (8) that relies on the attachment of DNA molecules to micro-sized surface structures in such a fashion that a large number of DNA molecules are stretched and lined up side by side (Fig. 1). Such so-called DNA curtains allow them to visualize a large number of individual molecules moving along DNA in a single experiment. During the first step of the MMR process, they observe that MutS $\alpha$  binds to the mismatch site after a combination of 1D sliding (with the protein maintaining contact with the DNA and rotating around it) and 3D diffusion. They also observe that MutS $\alpha$  can be released from the mismatch site by injection of ATP into their flow cell. This release is then followed by a more rapid diffusion that is more consistent with the protein moving along DNA without rotation. According to their data obtained with ATP- $\gamma$ S, only ATP binding and not hydrolysis is required to induce the release of MutS $\alpha$  from the mismatch.

Consistent with earlier work (9–11), the lifetime of the protein on DNA before mismatch binding depends strongly on ionic strength but becomes independent of salt concentration after release. This observation leads to the conclusion that the electrostatic interactions between the protein and DNA are modified, likely owing to conformational changes. The authors also discover that after ATP binding and mismatch release, MutS $\alpha$  no longer recognizes the mismatch site. This observation is consistent with the requirement to avoid a competitive trapping process of the protein on the mismatch after it has recognized the site and the system needs to proceed with the next steps of the repair process.

The authors' results are strongly in favor of the molecular switch model, in which MutS $\alpha$  scans the helical backbone of the

DNA to find the mismatch. Once the mismatch is found, MutS $\alpha$  binds ATP, which induces conformational changes that result in the formation of a clamp. As a ring, MutS $\alpha$  can then be released from the mismatch and slide along DNA

## The article by Gorman et al. represents a significant step forward in the characterization and understanding of DNA MMR.

without the strong interactions with the backbone that were required for the mismatch searching step.

The authors then proceed to study the interactions between MutS $\alpha$  and MutL $\alpha$ . Studying in real time the interactions between multiple proteins in a complex, multistep pathway is one of the strengths of single-molecule approaches, and in this case such an approach allows the authors to significantly increase our understanding of how the different partners in the MMR system act together. In particular, they observe that MutL $\alpha$  only colocalizes with MutS $\alpha$  on DNA containing a mismatch and only when MutS $\alpha$  was already bound to the mismatch. MutL $\alpha$  does not show any specificity for the mismatch by itself or for MutS $\alpha$  in other conditions. In contrast to the rotational sliding that MutL $\alpha$  uses to find the mismatch, MutS $\alpha$  targets it by using 1D hopping. Once ATP is added in the assay, the MutS $\alpha$ /MutL $\alpha$  complex escapes from the mismatch 1D diffusion with the same diffusion coefficient as for MutS $\alpha$  alone in the same context. Further, the authors show that the complex MutS $\alpha$ /

MutL $\alpha$  becomes resistant to increase in salt concentration after ATP binding, strengthening the conclusion that significant conformational changes occur upon ATP binding.

To also check whether intersegmental transfer might be involved in the various search processes underlying MMR, the authors design nanofabricated patterns that allow them to form well-defined crossings between two mismatch-containing DNA substrates with a distance between the crossing DNA molecules of  $\sim 100$  nm. They visualize trajectories of MutL $\alpha$  alone displaying  $90^\circ$  turns at the DNA intersections but do not observe such interstrand transfers for MutS $\alpha$ . This result suggests that intersegmental transfer can be involved in the search of mismatch-bound MutS $\alpha$  by MutL $\alpha$  but that the MutS $\alpha$ /MutL $\alpha$  complex needs to slide along the same DNA molecule so that it can scan flanking sequences searching for a cleavage site.

The article by Gorman et al. (1) represents a significant step forward in the characterization and understanding of DNA MMR. Their observation of an intricate hierarchy of search strategies supports the notion of a role for 1D diffusion along DNA as a physiologically important mechanism to speed up search. This work is a beautiful example of how single-molecule techniques have become more and more powerful in reconstituting complex biochemical pathways and probing the kinetic properties of the different steps. Extending these experiments to probe the interplay between the MMR process and the various components of the replication fork would represent the next step of understanding how these processes occur in a physiologically relevant context and would aid in bridging the gap between in vitro biochemistry and biophysics on the one hand and in vivo cell biology on the other.

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